

Applicant : Ernest G. Hopwood, Jr.  
Serial No. : 09/382,088  
Filed : August 24, 1999  
Page : 14

Attorney's Docket No.: 12932-003001

REMARKS

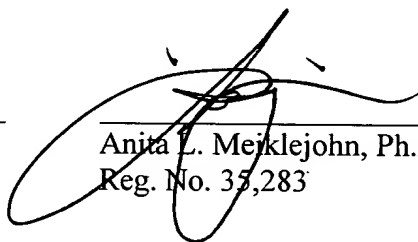
Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments to the specification and the claims merely correct typographical errors, insert sequence identifiers, and insert the paper copy of the substitute Sequence Listing in the specification. I hereby state that this submission, filed in accordance with 37 CFR §1.821(g), does not contain new matter.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 27 APRIL 2001

  
Anita L. Meiklejohn, Ph.D.  
Reg. No. 35,283

Fish & Richardson P.C.  
225 Franklin Street  
Boston, MA 02110-2804  
Telephone: (617) 542-5070  
Facsimile: (617) 542-8906

**Version with markings to show changes made**

**In the specification:**

Paragraph beginning at page 4, line 25 has been amended as follows:

In a preferred embodiment, cells, tissue or organs are contacted with an immunoprotecting amount of an Hsp47-related immunoprotective polypeptide. In one aspect, the polypeptide comprises the sequence AX<sub>1</sub>X<sub>2</sub>X<sub>3</sub>AX<sub>4</sub>X<sub>5</sub>X<sub>6</sub>R (SEQ ID NO:1). In this preferred embodiment, X<sub>1</sub> is V, L, A or T, X<sub>2</sub> is L or H, X<sub>3</sub> is S or V, X<sub>4</sub> is D or E, X<sub>5</sub> is Q, K or R, and X<sub>6</sub> is L or V. In another aspect, the polypeptide comprises the sequence AX<sub>1</sub>X<sub>2</sub>X<sub>3</sub>AEQLR (SEQ ID NO:29), where X<sub>1</sub>, X<sub>2</sub> and X<sub>3</sub> can be any amino acid. In a particularly preferred embodiment the polypeptide comprises the sequence AVLSAEQLR (SEQ ID NO:3). Polypeptides containing these sequences include the Hsp47 polypeptide disclosed herein as well as contain HLA-A antigens and IL-12 molecules. In a further embodiment, the method comprises contacting the tissue or organs with an immunoprotecting amount of a composition comprising brefeldin. Examples of the diseased states which cause the immune mediated damage include various autoimmune disease, graft vs. host disease and host vs. graft disease.

Paragraph beginning at page 5, line 15 has been amended as follows:

Figure 1 shows the nucleotide sequence and corresponding amino acid sequence of the human Hsp47 cDNA and protein, respectively (SEQ ID NOs:5 and 6, respectively). The complimentary strand is also shown (SEQ ID NO:4).

Paragraph beginning at page 5, line 17 has been amended as follows:

Figure 2 discloses related sequences among Hsp47 (human - SEQ ID NO:3; rat, mouse, and hamster - SEQ ID NO:8; chicken - SEQ ID NO:9), HLA-A antigens (SEQ ID NO:7) and IL-12 (human and mouse - SEQ ID NO:10; cat - SEQ ID NO:11; cow - SEQ ID NO:12) as well as consensus sequences (SEQ ID NOs:13, 27, 14, and 28, respectively).

Paragraph beginning at page 5, line 29 has been amended as follows:

Figures 8A and 8B shows the cloning of a huHsp47 gene cassette via PCR directed introduction of cloning sites and electrophoretic separation of expression products (SEQ ID NOs:15-18).

Paragraph beginning at page 6, line 7 has been amended as follows:

Figure 12 shows that the peptide "AVLSAEQLR" (SEQ ID NO:3) which is shared by both Hsp47 and HLA-A2\*201 protects HUVEC cells from CIK-mediated lysis.

Paragraph beginning at page 7, line 4 has been amended as follows:

In one aspect of the invention, the Hsp47-related immunoprotective polypeptide additionally is characterized by an amino acid sequence motif which corresponds to the overall consensus sequence as set forth in Figure 1. This can be represented as  $AX_1X_2X_3AX_4X_5X_6R$  (SEQ ID NO:1). In a preferred embodiment,  $X_1$  is V, L, A or T,  $X_2$  is L or H,  $X_3$  is S or V,  $X_4$  is D or E,  $X_5$  is Q, K or R, and  $X_6$  is L or V. This can alternatively be represented as  $A(v,l,a,t)(l,h)(s,v)A(d,e)(k,q,r)(l,v)R$  (SEQ ID NO:1).

Paragraph beginning at page 7, line 10 has been amended as follows:

An Hsp47-related immunoprotective polypeptide can also comprise an IL-12 consensus sequence such as that shown in Figure 2. This can be represented as  $AX_1LSAEX_5X_6R$  (SEQ ID NO:14) where  $X_1$  is preferably V, L or T,  $X_5$  is preferably Q, K or R, and  $X_6$  is L or V. This aspect of the invention can also be represented as  $A(v,l,t)LSAE(q,k,r)(l,v)R$  (SEQ ID NO:14).

Paragraph beginning at page 7, line 15 has been amended as follows:

In still a further aspect of the invention, the Hsp47-related immunoprotective polypeptide comprises the [consensus] consensus sequence as set forth for HLA-A as set forth in Figure 1. This can be represented as  $AX_1X_2X_3AEQLR$  (SEQ ID NO:2). In this embodiment,  $X_1$  is preferably V or A,  $X_2$  is preferably L or H,  $X_3$  is preferably S or V. This aspect of the invention can also be represented as  $A(v,a)(l,h)(s,v)AEQLR$  (SEQ ID NO:2).

Paragraph beginning at page 7, line 20 has been amended as follows:

In a particularly preferred aspect of the invention, the Hsp47-related polypeptide comprises the consensus sequence for Hsp as set forth in Figure 2. This can be represented as AVLSAX<sub>4</sub>X<sub>5</sub>LR (SEQ ID NO:13). In this embodiment, X<sub>4</sub> is preferably D or E, and X<sub>5</sub> is preferably K or Q. This aspect of the invention can also be represented as AVLSA(d,e)(k,q)LR (SEQ ID NO:13).

Paragraph beginning at page 7, line 25 has been amended as follows:

In still another preferred embodiment, the Hsp47-related immunoprotective polypeptide has the sequence AX<sub>1</sub>X<sub>2</sub>X<sub>3</sub>AEQLR (SEQ ID NO:29), wherein X<sub>1</sub>, X<sub>2</sub> and X<sub>3</sub> can be any amino acid and Hsp47 polypeptides preferably comprising the sequence AVLSAEQLR (SEQ ID NO:3).

Paragraph beginning at page 21, line 1 has been amended as follows:

The examples disclose the effect of brefeldin A and Hsp47 polypeptides including the polypeptide AVLSAEQLR (SEQ ID NO:3) on CIK cell lysis of cultures of endothelial cells derived from human umbilical cord samples. These experiments demonstrated that CIK lysis of the endothelial cells is inhibited by brefeldin A and by at least Hsp47 which is expressed upon contacting the endothelial cells with brefeldin A. The following discussion summarizes the examples set forth herein as well as other experimental results. However, such results are merely exemplary of the scope of the invention in that the methods and compositions of the invention can be used to meliorate not only the undesirable lysis of endothelial cells but immune mediated damage to other cells, tissues and organs. Moreover, the effect of such compositions and methods is not limited to CIK cells but rather to non-MHC I restricted cytotoxic T lymphocytes and natural killer cells in general. In this regard, it should also be noted that while the methods and compositions of the invention inhibit non-MHC I restricted CTLs, other modes of immune response are not significantly affected. In this regard, a BMT experiment was performed on full body irradiated mice treated with or without an Hsp47 polypeptide. Graft vs. host disease appeared in the non-Hsp47 treated group. Of the members of the Hsp47 treated group, none

developed graft vs. host disease. Moreover, none developed opportunistic infection over the time period tested which otherwise would have been expected if the entire immune system was suppressed.

Paragraph beginning at page 25, line 18 has been amended as follows:

BFA treatment has been reported to lead to a complex series of changes in treated cells, via selective intoxication of a G-protein responsible for protein transport between cis-and medial golgi compartments. This transport block results in a backing-up of secretion directed proteins into an enlarging ER system and leads finally to the fusion of the cis-golgi compartments with the ER (Lippincott-Schwartz *et al.*, *Cell* 56:801-813 (1989)). The enlargement of the ER compartment increases the synthesis of ER resident proteins. ER resident proteins are characterized by a C-terminal four amino acid "KDEL" (SEQ ID NO:19) or "RDEL" (SEQ ID NO:20)-ER retention signal. Examples of such ER-resident proteins are Hsp47, calreticulin, Grp78 and Grp94 (Ferreira *et al.*, *Arch. Virol.* 138:273-285 (1994); Ferreira *et al.*, *J. Cell Biochem.* 56:518-526 (1994); Ferreira *et al.*, *Connect Tissue Res.* 33:265-273 (1996); Smith *et al.*, *J. Biol. Chem.* 270:18323-18328 (1995)). These ER-resident proteins are not secreted out of the ER compartment, because they are in a  $\text{Ca}^{2+}$  dependent fashion recognized, bound to and recycled back into the ER by the KDEL/RDEL-receptors; Erd2.1 and Erd2.2. The ER serves as major intracellular  $\text{Ca}^{2+}$  store. Enlargement of the ER by BFA treatment perturbs the cellular  $\text{Ca}^{2+}$  homeostasis and leads to inactivation of the KDEL/RDEL receptor (Llewellyn *et al.*, *Biochemical and Biophysical Research Communications* 240:36-40 (1997)). ER resident proteins, including Hsp47, thus become freely secreted (Hu *et al.*, *J. Cell Biochem.* 59:350-367 (1995); Hu *et al.*, *J. Cell Biochem.* 59:214-234 (1995)). BFA treatment of EC therefore leads to an increased production of Hsp47 and to the free secretion of this otherwise ER resident protein. Under physiologic conditions, the transport of Hsp47 to the outside of the cell is linked to the expression and co-transport of chaperoned collagens I and IV (Yamamura *et al.*, *Biochem Biophys. Res. Comm.* 244:68-74 (1998)).

Paragraph beginning at page 27, line 26 has been amended as follows:

Human *huhsp47* was cloned without its signal peptide "MRSLLLGTLCLLAVALA" (SEQ ID NO:21). pGEX-4T bacterial protein expression system was used for IPTG induction to overexpress Hsp47 and to obtain a fusion protein of human Hsp47 and N-terminally tagged glutathione-S-transferase (GST). The latter allowed use of a one-step affinity purification protocol on GST substrate columns with crosslinked reduced glutathione and elution through competitive binding of this fusion partner with free, soluble reduced glutathione. GST itself has been shown to be non-toxic in most cellular assays and did not increase or reduce target cytotoxicity in the  $^{51}\text{Cr}$  release assays. Purified recombinant huHsp47 protects EC in a dose responsive fashion.

Paragraph beginning at page 28, line 3 has been amended as follows:

A Kozak initiation sequence and a functional secretion signal for optimal eukaryotic expression was introduced by a combination of PCR and restriction enzyme cloning, using the eukaryotic baculovirus protein expression vector pMel-Bac as template for the Kozak and the mellitin secretion leader sequences. Selection of the strong CMV<sup>IE</sup> promoter was done to ensure a high level of protein expression. The BFA treatment of EC resulted in a marked increase of secreted, extracellular Hsp47, despite the presence of an ER-retention signal "RDEL" (SEQ ID NO:20) on Hsp47. As detailed above, BFA treatment leads to  $\text{Ca}^{2+}$  flux [perturbation] perturbation of the ER compartment which results in dysfunctional ER retention of KDEL/RDEL-proteins (SEQ ID NOs:19 and 20), including Hsp47. To direct Hsp47 towards secretion in absence of BFA treatment, we deleted the "RVEL" (SEQ ID NO:22) sequence of Hsp47 in one of the constructs. Consistent with this concept, transfection of this construct results in increased secretion of Hsp47 protein and a higher (total) level of protection of EC from CIK, than mediated by the mostly ER retained form which only becomes transported to the cell surface via co-transport with collagen types I and IV (Hughes *et al.*, *Eur. J. Biochem.* 163:57-65 (1987)). The expression of Hsp47 and procollagen I were found to be tightly linked (Clarke *et al.*, *J. Cell Biol.* 121:193-199 (1993)).

Paragraph beginning at page 29, line 25 has been amended as follows:

The second domain is the short C-terminal ER retention signal ["RDEL."] "RDEL" (SEQ ID NO:20) which we discussed above.

Paragraph beginning at page 37, line 14 has been amended as follows:

Partial huHsp47 gene fragments derived by RT-PCR (a kind gift of Drs. Sanwal, Ontario, Canada) were amplified *in vitro* and artificial cloning sites introduced by PCR. These fragments correspond to nucleotides in Figure [1A] 1. The nucleic acid encoding the amino terminal 39 amino acids of Hsp47 (excluding the signal sequence and first amino acid of the mature protein) was amplified with the following primers: 5' primer ACGTTTGGATCCAGGTGAAGA (SEQ ID NO:15), 3' primer GTCCTTGGCCAT (SEQ ID NO:16). The 5' primer incorporated a Bam HI site to facilitate cloning into further vectors. The 3' primer incorporated an Mlu NI site to facilitate fusing the nucleic acids encoding the amino and the carboxy terminal portion of the protein. The nucleic acid encoding the carboxy terminal 360 amino acids was amplified with the following primers: 5' primer GCAATGGCCAAGGACCAGGCAGTGGAG (SEQ ID NO:17), 3' primer [ACGCTCTGCTCAATATCCTTAAGTCTA] ATCTGAATTCCTATAACTCGTCTCGCA (SEQ ID NO:18). The 5' primer incorporated an Mlu NI site to facilitate fusing the two portions of the gene into one continuous reading frame and the 3' primer incorporated an Eco RI site to facilitate cloning into further vectors. Standard PCR conditions generally known to those of skill in the art were used to amplify the two fragments from the starting clones (see Figure 8). The resulting amplification products were digested with Mlu NI, purified and ligated to each other. The resulting nucleic acid was further digested with Bam HI and Eco RI and the huHsp47 gene cassette was directionally cloned using standard recombinant DNA techniques into pUC 19 (Pharmacia, Uppsala, Sweden) which was used for construction purposes. The resulting plasmid, pUC/huHsp47, was amplified in recA bacterial host strain DH5a (Life Technologies, NY). The sequence of the cloned gene cassette was verified via fluorescent sequencing on an ABI sequencer (ABI,) using T7 DNA polymerase (Pharmacia, Uppsala, Sweden).

Paragraph beginning at page 38, line 28 has been amended as follows:

To dissect the role each domain of huHsp47 plays in protecting EC from CIK induced cytotoxicity, domain specific deletion mutants of huHsp47 were generated via PCR with nested primers based on the sequence disclosed in Figure 1 and previously published huHsp47 sequences. HuHsp 47 has at least four distinct functional domains. Beginning at the amino-terminus of the protein they are: a collagen/RGD binding domain, a domain with homology to the -2 domain of human HLA-A2 molecules, a serine protease inhibitor (serpin) domain and an ER retention signal RDEL (SEQ ID NO:20) domain. Three specific deletions were generated. Deletion 1 removed the RDEL (SEQ ID NO:20) domain, deletion 2 removed the RDEL (SEQ ID NO:20) and serpin domains and deletion 3 removed the carboxy-terminal 150 amino acids including the serpin and RDEL (SEQ ID NO:20) domains. The same 5' primer was used in PCR reactions to generate all three deletion mutants. The 5' primer sequence is: CGGAATTCTGGCCGAGGTGAAGAAACC (SEQ ID NO:23). The 3' primer used to generate the RDEL (SEQ ID NO:20) deletion mutant was the same primer used to generate the huHsp47-GFP fusion protein and has the sequence: AGTTCCCACTGTTCTACGACCTAGGGC (SEQ ID NO:24). The deletion 2 3' primer is: AACTCAACCTGTGTCTAGACCTATGGGC (SEQ ID NO:25). The deletion 3 3' primer is: ACGCGCTGCTCCTCCACGACCTAGGGC (SEQ ID NO:26) (see Figure 14). The 5' and 3' primers incorporated BamHI and EcoRI restriction enzyme sites respectively to facilitate subsequent cloning steps. The 5' and individual 3' primers were mixed with the pUC/huHsp47 plasmid containing the huHsp47 gene cassette and nucleic acids encoding each deletion mutant were PCR amplified in separate reactions. The PCR products were purified, digested with BamHI and EcoRI and ligated into either the pGEX-4T<sub>1</sub> vector to create huHsp47deletion-GST fusion proteins for cytotoxicity assays (see below) or pEGFP-N1, which also contained the mel secretion signal described above, to create eGFP-Hsp47deletion fusion proteins for use as FACS probes.

Paragraph beginning at page 44, line 1 has been amended as follows:

Subsequent to <sup>51</sup>Cr [labelling] labeling but prior to addition of effector cells 10-200 g FPLC purified Hsp47 (Example 7) protein or 10 nM to 10 M HPLC purified peptide AVLSAEQLR (SEQ ID NO:3) (Example 8), both in sterile PBS at pH 7.4 and diluted to the



final concentration with RPMI, were added to targets per ml of medium and incubated for 30 minutes at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>. In assays using purified protein, targets were subjected to three subsequent washes with 10 volumes of Ca<sup>2+</sup>, Mg<sup>2+</sup> and 5% FCS containing PBS prior to providing fresh standard culture medium to the target cells. In assays using purified peptide, the added peptide was present for the entire assay. The remainder of the assay was performed as detailed above, at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>.

Paragraph beginning at page 44, line 15 has been amended as follows:

The addition of as little as 1 μM of huHsp47-GST protein was able to reduce EC lysis by 50%. Pre-incubation with 10 μM of huHsp47-GST protein completely protects EC from CIK mediated lysis (see Figure 9). The peptide AVLSAEQLR (SEQ ID NO:3) was also able to protect EC from CIK mediated lysis in a dose dependent manner in assays where the E:T ration was 20:1 (see Figure 12). Peptide at a concentration of 1 μM was able to reduce EC lysis by 50% and peptide at a concentration of 10 μM was able to suppress CIK mediated lysis almost completely. The peptide was capable of reducing specific target lysis by less than 10% when used at 10 μM in a CIK-OCI-Ly8 tumor cell <sup>51</sup>Cr cytotoxicity assay with an E:T ratio of 20:1.

Paragraph beginning at page 44, line 24 has been amended as follows:

The protective effects of each of the huHsp47 deletion mutants (Fig. 15B) were also [analysed] analyzed. See Fig. 15C. Deletion 1 mutant protein, lacking the RDEL (SEQ ID NO:20) domain, reduced CIK mediated lysis by 40% indicating that the RDEL (SEQ ID NO:20) domain is not involved in protecting the EC from cytotoxicity. Deletion 2 mutant protein, lacking both the serpin and RDEL (SEQ ID NO:20) domains, only reduced CIK mediated lysis by 20% suggesting either the serpin domain is involved in protecting EC from cytotoxicity or the resulting protein is folded [aberrantly] aberrantly resulting in a less active form of the protein. Deletion 3 mutant protein, lacking the carboxy-terminal 150 amino acids, was as effective as deletion 1 mutant protein in protecting EC from CIK mediated lysis suggesting that the reduced protection seen with the deletion 2 mutant protein is due to an altered conformation of the protein and is not due to the serpin domain participating in the protection. The deletion 3 mutant protein

retains both the collagen/RGD binding domain and the  $\alpha 2$ -HLA-A2 homology domain which contains the AVLSAEQLR (SEQ ID NO:3) peptide sequence.

Paragraph beginning at page 45, line 7 has been amended as follows:

Nucleic acids encoding huHsp47 and fragments were cloned into [eucaryotic] eukaryotic expression vectors. A nucleic acid encoding a fragment of huHsp47, in which the carboxy-terminal RDEL (SEQ ID NO:20) amino acid sequence is deleted, was PCR amplified from the pUC/huHsp47 plasmid using the following primers: 5' primer CGGAATTCTGGCCGAGGTGAAGAAACC (SEQ ID NO:23), 3' primer AGTTCCCACTGTTCTACGACCTAGGGC (SEQ ID NO:24). The amplified product was ligated to the melittin secretion signal and Kozak sequences derived from pMel-Bac (Invitrogen, San Diego, CA) and the resulting fragment was cloned into the multiple cloning site of pEGFP-N1 (Clontech, Palo Alto, CA) using general techniques well known to those of skill in the art. The resulting plasmid, eGFP-Hsp47, was transfected into EC.

Paragraph beginning at page 46, line 3 has been amended as follows:

EC targets were used in cytotoxicity assays 24 hours after lipofectamine mediated transfection of either CMV-neo-mel-huHsp47fulllength or CMV-neo-mel huHsp47 $\delta$ RDEL into previously susceptible EC. Mature d<sub>21</sub> CIK cells were used at an E:T ratio of 20:1. EC over-expressing full length huHsp47 showed a 25% reduction in the CIK mediated lysis. EC over-expressing huHsp47 $\delta$ RDEL were entirely resistant to CIK mediated lysis (see Figure 11). Deletion of the RDEL peptide (SEQ ID NO:20), which serves as an endoplasmic reticulum (ER) retention signal, from huHsp47, allows the protein to be freely secreted and protects the EC targets expressing the protein from CIK mediated lysis.

Paragraph beginning at page 53, line 8 has been amended as follows:

A hydrophilic 9 mer peptide of the sequence AVLSAEQLR (SEQ ID NO:3) which corresponds to the region of shared homology of Hsp47 with HLA-A2 was synthesized by the Stanford PAN facility on a BioApplied® peptide synthesizer, HPLC purified and verified by

amino acid sequencing. Pure peptide was lyophilized and stored at -20 °C. The peptide was soluble in aqueous solution and used in PBS and RPMI 1640 at indicated concentrations.

In the claims:

Claims 2-5, 9-13, 20-23, 27-30, 33, 36, and 37 have been amended as follows:

2. (Amended) The method of Claim 1 wherein said Hsp47-related immunoprotective polypeptide comprises the sequence  $AX_1X_2X_3AX_4X_5X_6R$  (SEQ ID NO:1) wherein  $X_1$  is V, L, A or T,  $X_2$  is L or H,  $X_3$  is S or V,  $X_4$  is D or E,  $X_5$  is Q, K or R, and  $X_6$  is L or V.

3. (Amended) The method of Claim 1 wherein said Hsp47-related immunoprotective polypeptide comprises the sequence  $AX_1X_2X_3AEQLR$  (SEQ ID NO:29), where  $X_1$ ,  $X_2$  and  $X_3$  can be any amino acid.

4. (Amended) The method according to Claim 3 wherein  $X_1$  is V or A,  $X_2$  is L or H and  $X_3$  is S or V (SEQ ID NO:2).

5. (Amended) The method according to Claim 1 wherein said polypeptide comprises the sequence  $AVLSAEQLR$  (SEQ ID NO:3).

9. (Amended) The method according to Claim 8 wherein said Hsp47 polypeptide comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence shown in Figure 1 (SEQ ID NO:6);
- (b) an amino acid sequence having at least about 70% amino acid sequence identity with the amino acid sequence of (a);
- (c) an immunoprotecting fragment of (a) or (b); and
- (d) an immunoprotecting variant of (a) or (b).

10. (Amended) The method according to Claim 9 wherein said immunoprotecting fragment of Hsp 47 comprises the amino acid sequence  $AVLSAEQLR$  (SEQ ID NO:3).

11. (Amended) The method according to Claim 9 wherein said immunoprotecting variant of Hsp 47 is a conserved variant of the sequence AVL<sup>S</sup>AEQLR (SEQ ID NO:3).

12. (Amended) The method according to Claim 9 wherein said immunoprotecting variant of Hsp 47 comprises the sequence AAHV<sup>A</sup>AEQLR (SEQ ID NO:7).

13. (Amended) The method according to Claim 8 wherein said Hsp47 polypeptide comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence shown in Figure 1 (SEQ ID NO:6);
- (b) an amino acid sequence encoded by a nucleic acid that hybridizes to a nucleic acid that encodes the amino acid sequence shown in Figure 1 (SEQ ID NO:5);
- (c) an immunoprotecting fragment of (a) or (b); and
- (d) an immunoprotecting variant of (a) or (b).

20. (Amended) The method according to Claim 19 wherein said Hsp47-related immunoprotective polypeptide comprises the sequence AX<sub>1</sub>X<sub>2</sub>X<sub>3</sub>AX<sub>4</sub>X<sub>5</sub>X<sub>6</sub>R (SEQ ID NO:1) wherein X<sub>1</sub> is V, L, A or T, X<sub>2</sub> is L or H, X<sub>3</sub> is S or V, X<sub>4</sub> is D or E, X<sub>5</sub> is Q, K or R, and X<sub>6</sub> is L or V.

21. (Amended) The method according to Claim 19 wherein said Hsp47-related immunoprotective polypeptide comprises the sequence AX<sub>1</sub>X<sub>2</sub>X<sub>3</sub>AEQLR (SEQ ID NO:29), where X<sub>1</sub>, X<sub>2</sub> and X<sub>3</sub> can be any amino acid.

22. (Amended) The method according to Claim 21 wherein X<sub>1</sub> is V or A, X<sub>2</sub> is L or H and X<sub>3</sub> is S or V (SEQ ID NO:2).

23. (Amended) The method, according to Claim 19, wherein said polypeptide comprises the sequence AVL<sup>S</sup>AEQLR (SEQ ID NO:3).

27. (Amended) The method according to Claim 26 wherein said immunoprotective polypeptide comprises the sequence  $AX_1X_2X_3AX_4X_5X_6R$  (SEQ ID NO:1) wherein  $X_1$  is V, L, A or T,  $X_2$  is L or H,  $X_3$  is S or V,  $X_4$  is D or E,  $X_5$  is Q, K or R, and  $X_6$  is L or V.

28. (Amended) The method of Claim 26 wherein said immunoprotective polypeptide comprises the sequence  $AX_1X_2X_3AEQLR$  (SEQ ID NO:29), where  $X_1$ ,  $X_2$  and  $X_3$  can be any amino acid.

29. (Amended) A method according to Claim 28 wherein  $X_1$  is V or A,  $X_2$  is L or H and  $X_3$  is S or V (SEQ ID NO:2).

30. (Amended) A method for identifying cells which bind to a polypeptide comprising contacting said cells with a polypeptide comprising the sequence  $AX_1X_2X_3AX_4X_5X_6R$  (SEQ ID NO:1) wherein  $X_1$  is V, L, A or T,  $X_2$  is L or H,  $X_3$  is S or V,  $X_4$  is D or E,  $X_5$  is Q, K or R, and  $X_6$  is L or V;  
wherein said polypeptide further comprises a detectable label, and  
detecting cells which bind said labeled polypeptide.

33. (Amended) An Hsp47 polypeptide comprising the sequence  $AVLSAEQLR$  (SEQ ID NO:3) and a label.

36. (Amended) An antibody which specifically binds to an Hsp47 polypeptide epitope comprising the sequence  $AX_1X_2X_3AEQLR$  (SEQ ID NO:29).

37. (Amended) An anti-idiotypic antibody to a polypeptide comprising the sequence  $AVLSAEQLR$  (SEQ ID NO:3).